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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/620,777	07/15/2003	Roy Curtiss III	56029/	1127

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EXAMINER	
GANGLE, BRIAN J	

ART UNIT	PAPER NUMBER
1645	

DATE MAILED: 11/14/2006

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/620,777	CURTISS ET AL.	
	Examiner	Art Unit	
	Brian J. Gangle	1645	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *07 August 2006*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *61-107* is/are pending in the application.

4a) Of the above claim(s) *65,66,68,69,74-82 and 95-103* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *61-64, 67, 70-73, 83-94, and 104-107* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *1/31/05*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Election/Restrictions

Applicant's election with traverse of Group I, where the *asd* is extrachromosomal, in the response filed 8/7/2006 is acknowledged. The traversal is on the ground(s) that the examiner has not shown the products to be distinct and has not shown that examination of all of the claims would be a burden.

Applicant argues:

1. That the restriction requirement in the parent case only contained 2 groups and that it is inconsistent to now have 19 groups.

2. That to support restriction between related products, it must be shown that the inventions, as claimed, do not overlap in scope, are not obvious variants, and are not capable of use together or can have a materially different design, mode of operation, function, or effect. Applicant argues that the claimed products overlap, and are capable of use together. Applicant states that the claimed products are all environmentally limited viability systems for microbes, and that the products do not have materially different designs, modes of operation, functions, or effects. Applicant states that these microorganisms are limited to permissive environments by specifically expressing different genes in different environments.

3. That it has not been shown that it would be a burden for the examiner to examine all of the inventions.

Applicant's arguments have been fully considered and deemed non-persuasive.

Regarding argument 1, the original restriction requirement in the parent case was later revised, and the claims were subject to a restriction requirement containing **54** groups. Further, the instant application is a Continuation-in-part and contains a different claim set than the one originally filed.

Regarding argument 2, the inventions, as claimed meet all of the requirements for restriction. The product groups do not overlap in scope. For example, the cells of Group I contains an essential gene, while the cells of Group II contain a lethal gene, and Group III contains cells with both an essential gene and a lethal gene. The cells of any of these groups would not fit into the other groups. Neither are these groups obvious variants. These cells have different designs, modes of operation, functions, and effects. A cell with a lethal gene has a

completely different design, mode of operation, function, and effect than a cell without a lethal gene. Moreover, applicant has mischaracterized the claimed microbial cells by stating that these microorganisms are limited to permissive environments by specifically expressing different genes in different environments. The groups do not contain cells that are different because of differential expression of particular genes. The claimed cells must contain specific genes. A cell that contains a lethal gene and does not express it is entirely different from a cell that does not contain said lethal gene.

Regarding argument 3, the literature search, particularly relevant in this art, is not co-extensive, because, for example, a cell containing a lethal gene would not be found in a search for cells containing essential genes. Clearly, different searches and issues are involved in the examination of each Group.

The requirement is still deemed proper and is therefore made FINAL.

Claims 61-107 are pending. Claims 65-66, 68-69, 74-82, and 95-103 are withdrawn as being drawn to non-elected inventions. Claims 61-64, 67, 70-73, 83-94, and 104-107 are currently under examination.

Information Disclosure Statement

The information disclosure statement filed 1/31/2005 has been considered. An initialed copy is enclosed. Those references which are crossed out were unavailable and will be considered when they become available.

Claim Objections

Claims 61-64, 67, 70-73, 83-94, and 104-107 are objected to because of the following informalities: the claims are drawn, in part, to non-elected subject matter. Appropriate correction is required.

Double Patenting

The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or

improper timewise extension of the "right to exclude" granted by a patent and to prevent possible harassment by multiple assignees. See *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970);and, *In re Thorington,* 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) may be used to overcome an actual or provisional rejection based on a nonstatutory double patenting ground provided the conflicting application or patent is shown to be commonly owned with this application. See 37 CFR 1.130(b).

Effective January 1, 1994, a registered attorney or agent of record may sign a terminal disclaimer. A terminal disclaimer signed by the assignee must fully comply with 37 CFR 3.73(b).

Claims 61-62, 70-71, 73, 84, 88, 91-94, and 104-107 are rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 6-9 of U.S. Patent No. 5,840,483. Although the conflicting claims are not identical, they are not patentably distinct from each other because both claim sets are drawn to bacterial cells (Enterobacteriaceae) which lack a functional chromosomal *asd* gene and which contain said gene on an extrachromosomal vector, wherein said cells would be viable in a permissive environment, but non-viable in a non-permissive environment, and wherein the *asd* gene would be expressed in the permissive environment, but not in the non-permissive environment.

Claims 61-62, 70-73, 84, 88, 91-94, and 104-107 are rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 1, 3, and 7-8 of U.S. Patent No. 5,672,345. Although the conflicting claims are not identical, they are not patentably distinct from each other because both claim sets are drawn to bacterial cells (*Salmonella)* which lack a functional chromosomal *asd* gene and which contain said gene on an extrachromosomal vector, wherein said cells would be viable in a permissive environment, but non-viable in a non-permissive environment, and wherein the *asd* gene would be expressed in the permissive environment, but not in the non-permissive environment.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 61-64, 67, 73, 83-89, and 104-107 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

Applicant is directed to the Guidelines for the Examination of Patent Applications Under the 35 U.S.C. 112, first paragraph "Written Description" Requirement, Federal Register, Vol. 66, No. 4, pages 1099-1111, Friday January 5, 2001.

The specification discloses working examples using *Salmonella*. Said *Salmonella* contain a defined chromosomal *asd* deletion and a promoter bearing *cI*857 and lambda promoter which allows expression of the plasmid-borne *asd* essential gene. In addition, the specification provides an example of *Salmonella* that contain a defined chromosomal *asd* deletion, wherein expression of the plasmid-borne *asd* essential gene is regulated by the presence of arabinose. However, the aforementioned claims encompass all microbial cells, including bacteria, fungi, and protozoans, many of which are not known to contain *asd* as an essential gene. The specification provides insufficient written description to support the genus encompassed by the claims.

MPEP § 2163.02 states, "[a]n objective standard for determining compliance with the written description requirement is, 'does the description clearly allow persons of ordinary skill in the art to recognize that he or she invented what is claimed' ". The courts have decided:

> The purpose of the "written description" requirement is broader than to merely explain how to "make and use"; the applicant must convey with reasonable clarity to those skilled in the art that, as of the filing date sought, he or she was in possession *of the invention*. The invention is, for purposes of the "written

description" inquiry, *whatever is now claimed.*

See *Vas-Cath, Inc. v. Mahurkar*, 935 F.2d 1555, 1563-64, 19 USPQ2d 1111, 1117 (Federal Circuit, 1991). Furthermore, the written description provision of 35 USC § 112 is severable from its enablement provision; and adequate written description requires more than a mere statement that it is part of the invention and reference to a potential method for isolating it. See *Fiers v. Revel*, 25 USPQ2d 1601, 1606 (CAFC 1993) and *Amgen Inc. V. Chugai Pharmaceutical Co. Ltd.*, 18 USPQ2d 1016.

The Guidelines for Examination of Patent Applications Under the 35 U.S.C. 112, paragraph 1, ``Written Description'' Requirement (66 FR 1099-1111, January 5, 2001) state, "[p]ossession may be shown in a variety of ways including description of an actual reduction to practice, or by showing the invention was 'ready for patenting' such as by disclosure of drawings or structural chemical formulas that show that the invention was complete, or by describing distinguishing identifying characteristics sufficient to show that the applicant was in possession of the claimed invention" (*Id.* at 1104). Moreover, because the claims encompass a genus of variant species, an adequate written description of the claimed invention must include sufficient description of at least a representative number of species by actual reduction to practice, reduction to drawings, or by disclosure of relevant, identifying characteristics sufficient to show that Applicant was in possession of the claimed genus. However, factual evidence of an actual reduction to practice has not been disclosed by Applicant in the specification; nor has Applicant shown the invention was "ready for patenting" by disclosure of drawings or structural chemical formulas that show that the invention was complete; nor has Applicant described distinguishing identifying characteristics (such as the presence of *asd* as an essential gene) sufficient to show that Applicant were in possession of the claimed invention at the time the application was filed.

Therefore, only bacterial cells containing the claimed environmentally limited viability system meet the written description provision of 35 USC 112, first paragraph. The species specifically disclosed are not representative of the genus because the genus is highly variant. Applicant is reminded that Vas-Cath makes clear that the written description provision of 35 USC 112 is severable from its enablement provision. (See page 1115.)

Claims 61-64, 67, 73, 83-89, and 104-107 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for bacterial cells containing the claimed environmentally limited viability system, does not reasonably provide enablement for microbial cells containing the claimed environmentally limited viability system. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims.

Enablement is considered in view of the Wands factors (MPEP 2164.01(A)). These include: nature of the invention, breadth of the claims, guidance of the specification, the existence of working examples, state of the art, predictability of the art and the amount of experimentation necessary.

Nature of the invention: The instant claims are drawn to microbial cells that contain an environmentally limited viability system wherein the *asd* gene serves as an essential gene, without the expression of which, the cells will die.

Breadth of the claims: The claims are broadly drawn in that they encompass all microbial cells. This includes all microorganisms, many of which are eukaryotes.

Guidance of the specification/The existence of working examples: The specification provides working examples using *Salmonella*. Said *Salmonella* contain a defined chromosomal *asd* deletion and a promoter bearing *cI*857 and lambda promoter which allows expression of the plasmid-borne *asd* essential gene. In addition, the specification provides an example of *Salmonella* that contain a defined chromosomal *asd* deletion, wherein expression of the plasmid-borne *asd* essential gene is regulated by the presence of arabinose.

State of the art: The art shows that the *asd* gene encodes aspartate β-semialdehyde dehydrogenase, which is part of the biosynthetic pathway for synthesis of diaminopimelic acid, an essential component of the peptidoglycan of bacterial cell walls (Nakayama *et al.*, Bio/Technology, 6:693-697, IDS filed 1/31/2005; Galan *et al.*, Gene, 94:29-35, 1990). The art does not suggest that the *asd* gene is essential to organisms other than bacteria.

The claims encompass organisms, other than bacteria, which must have the essential *asd* gene. However, there is no guidance in the specification to show that this gene is essential to organisms other than bacteria, as only bacteria contain peptidoglycan in their cell walls.

Therefore, the claimed environmentally limited viability system is only possible in bacteria, and the full scope of the claims is not enabled.

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 61-64, 67, 70-73, 83-94, and 104-107 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claims 61, 84, and 87 are rendered vague and indefinite by the use of the word "temporarily." The specification defines the term "temporary" as "not permanent" and states that the use of this term is not limited to any specific period of time (page 14, lines 8-16). If the term is not limiting, then the term cannot be a limitation of the claim, and it is unclear what is intended by its use. Further, regarding the phrase "temporarily viable," since the term only means "not permanently viable," all cells would be considered "temporarily viable" because all cells die at some point.

Claims 61, 84, and 87-88 are rendered vague and indefinite by the use of the term "viable." The specification defines a "non-viable" cell as one that cannot grow. Does this mean that the definition of a "viable" cell is one that can grow? If so, does applicant intend a viable cell to be one which is currently growing, or one which is capable of growing? If the latter is the case, the only way for a cell to be non-viable is to be dead.

The term "about" in claims 85 and 89 is a relative term which renders the claim indefinite. The term "about" is not defined by the claim, the specification does not provide a standard for ascertaining the requisite degree, and one of ordinary skill in the art would not be reasonably apprised of the scope of the invention.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

A person shall be entitled to a patent unless –

(b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

(e) the invention was described in (1) an application for patent, published under section 122(b), by another filed in the United States before the invention by the applicant for patent or (2) a patent granted on an application for patent by another filed in the United States before the invention by the applicant for patent, except that an international application filed under the treaty defined in section 351(a) shall have the effects for purposes of this subsection of an application filed in the United States only if the international application designated the United States and was published under Article 21(2) of such treaty in the English language.

(e) the invention was described in a patent granted on an application for patent by another filed in the United States before the invention thereof by the applicant for patent, or on an international application by another who has fulfilled the requirements of paragraphs (1), (2), and (4) of section 371(c) of this title before the invention thereof by the applicant for patent.

The changes made to 35 U.S.C. 102(e) by the American Inventors Protection Act of 1999 (AIPA) and the Intellectual Property and High Technology Technical Amendments Act of 2002 do not apply when the reference is a U.S. patent resulting directly or indirectly from an international application filed before November 29, 2000. Therefore, the prior art date of the reference is determined under 35 U.S.C. 102(e) prior to the amendment by the AIPA (pre-AIPA 35 U.S.C. 102(e)).

Claims 61-62, 70-73, 83-84, 87-88, 91-94, and 104-107 rejected under 35 U.S.C. 102(b) as being anticipated by Galan *et al.* (Gene, 94:29-35, 1990).

The instant claims are drawn to an isolated microbial cell comprising an Environmentally Limited Viability System, wherein the cell is viable in a permissive environment and non-viable or temporarily viable in a non-permissive environment, the system comprising an essential gene (*asd*), wherein expression of the essential gene in the cell is essential to the viability of the cell, the essential gene is expressed when the cell is in the permissive environment and is not expressed or temporarily expressed when the cell is in the non-permissive environment (claim 61); wherein the cell of claim 61 wherein the cell grows in the permissive environment and dies in the non-permissive environment (claim 62); wherein the cell is a gram-negative bacterium (claim 70); wherein the gram-negative bacterium is an enteric bacterium (claim 71); wherein the

genus of the enteric bacterium is selected from the group consisting of Escherichia and Salmonella (claim 72); wherein the essential gene is carried on an extrachromosomal vector (claim 73); and wherein the essential gene has engineered expression (claim 83). Additional claims are drawn to a method of making a cell strain with environmentally limited viability comprising stably introducing into a cell an essential gene (*asd*), wherein expression of the essential gene in the cell is essential to the viability of the cell, the essential gene is expressed when the cell is in the permissive environment and is not expressed or temporarily expressed when the cell is in the non-permissive environment; wherein the cell strain is viable in a permissive environment and non-viable or temporarily viable in a non-permissive environment, and wherein the essential gene is essential for metabolism, growth, cell wall integrity, or cell membrane integrity of the cell (claim 84); wherein the cell is temporarily viable in the non-permissive environment (claim 87). Claims are also drawn to an isolated microbial cell comprising an Environmentally Limited Viability System, wherein the cell is viable in a permissive environment and non-viable in a non-permissive environment, the system comprising an essential gene, wherein expression of the gene in the cell is essential to the viability of the cell, and wherein said essential gene is expressed when the cell is in the permissive environment and is not expressed when the cell is in the non-permissive environment, and wherein said essential gene is essential for metabolism, growth, cell wall integrity, or cell membrane integrity of the cell (claim 88); wherein the essential gene is carried on an extrachromosomal vector (claim 91); wherein the cell is a gram-negative bacterium (claim 92); wherein the gram-negative bacterium is an enteric bacterium (claim 93); wherein the genus of the enteric bacterium is selected from the group consisting of Escherichia and Salmonella (claim 94); wherein the essential gene is a gene essential for metabolism or growth of the cell (claim 104); wherein the essential is a gene essential for cell wall or cell membrane integrity (claim 105); wherein the essential gene is a gene encoding an enzyme that catalyzes steps in the biosynthesis of DAP (claim 106); wherein the essential gene is an *asd* gene (claim 107).

Galan *et al.* disclose an *asd* mutant (and a method of making said mutant) of *Salmonella* wherein the chromosomal *asd* gene is deleted and the strain contains a plasmid with the *asd* gene (see abstract). The *asd* gene is connected to a P_{trc} promoter, which serves as a means of engineered expression (see page 32, column 1). The instant specification defines a permissive

environment as an environment in which the claimed cells are viable, and a non-permissive environment as one in which the cells are non-viable or temporarily viable. As all gene expression would cease when a cell dies (becomes non-viable), the essential gene (*asd*) of the cells disclosed by Galan *et al.* would not be expressed in a non-permissive environment. Hence, the cells disclosed by Galan *et al.* meet the limitations of the instantly claimed invention.

Claims 61-62, 70-73, 84, 88, 91-94, and 104-107 are rejected under 35 U.S.C. 102(e) as being anticipated by Curtiss (US Patent 5,840,483)

The applied reference has a common inventor with the instant application. Based upon the earlier effective U.S. filing date of the reference, it constitutes prior art under 35 U.S.C. 102(e). This rejection under 35 U.S.C. 102(e) might be overcome either by a showing under 37 CFR 1.132 that any invention disclosed but not claimed in the reference was derived from the inventor of this application and is thus not the invention "by another," or by an appropriate showing under 37 CFR 1.131.

The instant claims are drawn to an isolated microbial cell comprising an Environmentally Limited Viability System, wherein the cell is viable in a permissive environment and non-viable or temporarily viable in a non-permissive environment, the system comprising an essential gene (*asd*), wherein expression of the essential gene in the cell is essential to the viability of the cell, the essential gene is expressed when the cell is in the permissive environment and is not expressed or temporarily expressed when the cell is in the non-permissive environment (claim 61); wherein the cell of claim 61 wherein the cell grows in the permissive environment and dies in the non-permissive environment (claim 62); wherein the cell is a gram-negative bacterium (claim 70); wherein the gram-negative bacterium is an enteric bacterium (claim 71); wherein the genus of the enteric bacterium is selected from the group consisting of Escherichia and Salmonella (claim 72); wherein the essential gene is carried on an extrachromosomal vector (claim 73). Additional claims are drawn to a method of making a cell strain with environmentally limited viability comprising stably introducing into a cell an essential gene (*asd*), wherein expression of the essential gene in the cell is essential to the viability of the cell, the essential gene is expressed when the cell is in the permissive environment and is not expressed or temporarily expressed when the cell is in the non-permissive environment; wherein

the cell strain is viable in a permissive environment and non-viable or temporarily viable in a non-permissive environment, and wherein the essential gene is essential for metabolism, growth, cell wall integrity, or cell membrane integrity of the cell (claim 84). Claims are also drawn to an isolated microbial cell comprising an Environmentally Limited Viability System, wherein the cell is viable in a permissive environment and non-viable in a non-permissive environment, the system comprising an essential gene, wherein expression of the gene in the cell is essential to the viability of the cell, and wherein said essential gene is expressed when the cell is in the permissive environment and is not expressed when the cell is in the non-permissive environment, and wherein said essential gene is essential for metabolism, growth, cell wall integrity, or cell membrane integrity of the cell (claim 88); wherein the essential gene is carried on an extrachromosomal vector (claim 91); wherein the cell is a gram-negative bacterium (claim 92); wherein the gram-negative bacterium is an enteric bacterium (claim 93); wherein the genus of the enteric bacterium is selected from the group consisting of Escherichia and Salmonella (claim 94); wherein the essential gene is a gene essential for metabolism or growth of the cell (claim 104); wherein the essential is a gene essential for cell wall or cell membrane integrity (claim 105); wherein the essential gene is a gene encoding an enzyme that catalyzes steps in the biosynthesis of DAP (claim 106); wherein the essential gene is an *asd* gene (claim 107).

Curtiss discloses an *asd* mutant (and a method of making said mutant) of *Salmonella* wherein the chromosomal *asd* gene is deleted and the strain contains a plasmid with the *asd* gene (col. 9, line 25- col. 10. line 35; claims 6-9). The instant specification defines a permissive environment as an environment in which the claimed cells are viable, and a non-permissive environment as one in which the cells are non-viable or temporarily viable. As all gene expression would cease when a cell dies (becomes non-viable), the essential gene (*asd*) of the cells disclosed by Curtiss would not be expressed in a non-permissive environment. Hence, the cells disclosed by Curtiss meet the limitations of the instantly claimed invention.

Claims 61-62, 70-73, 84, 88, 91-94, and 104-107 are rejected under 35 U.S.C. 102(e) as being anticipated by Curtiss (US Patent 5,672,345)

The applied reference has a common inventor with the instant application. Based upon the earlier effective U.S. filing date of the reference, it constitutes prior art under 35 U.S.C.

102(e). This rejection under 35 U.S.C. 102(e) might be overcome either by a showing under 37 CFR 1.132 that any invention disclosed but not claimed in the reference was derived from the inventor of this application and is thus not the invention "by another," or by an appropriate showing under 37 CFR 1.131.

The instant claims are drawn to an isolated microbial cell comprising an Environmentally Limited Viability System, wherein the cell is viable in a permissive environment and non-viable or temporarily viable in a non-permissive environment, the system comprising an essential gene (*asd*), wherein expression of the essential gene in the cell is essential to the viability of the cell, the essential gene is expressed when the cell is in the permissive environment and is not expressed or temporarily expressed when the cell is in the non-permissive environment (claim 61); wherein the cell of claim 61 wherein the cell grows in the permissive environment and dies in the non-permissive environment (claim 62); wherein the cell is a gram-negative bacterium (claim 70); wherein the gram-negative bacterium is an enteric bacterium (claim 71); wherein the genus of the enteric bacterium is selected from the group consisting of Escherichia and Salmonella (claim 72); wherein the essential gene is carried on an extrachromosomal vector (claim 73). Additional claims are drawn to a method of making a cell strain with environmentally limited viability comprising stably introducing into a cell an essential gene (*asd*), wherein expression of the essential gene in the cell is essential to the viability of the cell, the essential gene is expressed when the cell is in the permissive environment and is not expressed or temporarily expressed when the cell is in the non-permissive environment; wherein the cell strain is viable in a permissive environment and non-viable or temporarily viable in a non-permissive environment, and wherein the essential gene is essential for metabolism, growth, cell wall integrity, or cell membrane integrity of the cell (claim 84). Claims are also drawn to an isolated microbial cell comprising an Environmentally Limited Viability System, wherein the cell is viable in a permissive environment and non-viable in a non-permissive environment, the system comprising an essential gene, wherein expression of the gene in the cell is essential to the viability of the cell, and wherein said essential gene is expressed when the cell is in the permissive environment and is not expressed when the cell is in the non-permissive environment, and wherein said essential gene is essential for metabolism, growth, cell wall integrity, or cell membrane integrity of the cell (claim 88); wherein the essential gene is carried on an

extrachromosomal vector (claim 91); wherein the cell is a gram-negative bacterium (claim 92); wherein the gram-negative bacterium is an enteric bacterium (claim 93); wherein the genus of the enteric bacterium is selected from the group consisting of Escherichia and Salmonella (claim 94); wherein the essential gene is a gene essential for metabolism or growth of the cell (claim 104); wherein the essential is a gene essential for cell wall or cell membrane integrity (claim 105); wherein the essential gene is a gene encoding an enzyme that catalyzes steps in the biosynthesis of DAP (claim 106); wherein the essential gene is an *asd* gene (claim 107).

Curtiss discloses an *asd* mutant (and a method of making said mutant) of *Salmonella* wherein the chromosomal *asd* gene is deleted and the strain contains a plasmid with the *asd* gene (col. 8, line 66- col. 10. line 11; claims 1, 3, and 7-8). The instant specification defines a permissive environment as an environment in which the claimed cells are viable, and a non-permissive environment as one in which the cells are non-viable or temporarily viable. As all gene expression would cease when a cell dies (becomes non-viable), the essential gene (*asd*) of the cells disclosed by Curtiss would not be expressed in a non-permissive environment. Hence, the cells disclosed by Curtiss meet the limitations of the instantly claimed invention.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Claims 61-63, 67, 70-73, 83-84, 87-88, 91-94, and 104-107 are rejected under 35 U.S.C. 103(a) as being unpatentable over Galan *et al.* (Gene, 94:29-35, 1990) in view of Guzman *et al.* (J. Bacteriol., 177:4121-4130, 1995).

The instant claims are drawn to an isolated microbial cell comprising an Environmentally Limited Viability System, wherein the cell is viable in a permissive environment and non-viable or temporarily viable in a non-permissive environment, the system comprising an essential gene (*asd*), wherein expression of the essential gene in the cell is essential to the viability of the cell, the essential gene is expressed when the cell is in the permissive environment and is not expressed or temporarily expressed when the cell is in the non-permissive environment (claim 61); wherein the cell of claim 61 wherein the cell grows in the permissive environment and dies in the non-permissive environment (claim 62); wherein the permissive environment comprises an environment containing a nutrient required to maintain expression of the essential gene and the non-permissive environment comprises an environment lacking the nutrient (claim 63); wherein the essential gene comprises the *asd* gene operatively linked to *araC*-Pbad (claim 67); wherein the cell is a gram-negative bacterium (claim 70); wherein the gram-negative bacterium is an enteric bacterium (claim 71); wherein the genus of the enteric bacterium is selected from the group consisting of Escherichia and Salmonella (claim 72); wherein the essential gene is carried on an extrachromosomal vector (claim 73); and wherein the essential gene has engineered expression (claim 83). Additional claims are drawn to a method of making a cell strain with environmentally limited viability comprising stably introducing into a cell an essential gene (*asd*), wherein expression of the essential gene in the cell is essential to the viability of the cell, the essential gene is expressed when the cell is in the permissive environment and is not expressed or temporarily expressed when the cell is in the non-permissive environment; wherein the cell strain is viable in a permissive environment and non-viable or temporarily viable in a non-permissive environment, and wherein the essential gene is essential for metabolism, growth, cell wall integrity, or cell membrane integrity of the cell (claim 84); wherein the cell is temporarily viable in the non-permissive environment (claim 87). Claims are also drawn to an isolated microbial cell comprising an Environmentally Limited Viability System, wherein the cell is viable in a permissive environment and non-viable in a non-permissive environment, the

system comprising an essential gene, wherein expression of the gene in the cell is essential to the viability of the cell, and wherein said essential gene is expressed when the cell is in the permissive environment and is not expressed when the cell is in the non-permissive environment, and wherein said essential gene is essential for metabolism, growth, cell wall integrity, or cell membrane integrity of the cell (claim 88); wherein the essential gene is carried on an extrachromosomal vector (claim 91); wherein the cell is a gram-negative bacterium (claim 92); wherein the gram-negative bacterium is an enteric bacterium (claim 93); wherein the genus of the enteric bacterium is selected from the group consisting of Escherichia and Salmonella (claim 94); wherein the essential gene is a gene essential for metabolism or growth of the cell (claim 104); wherein the essential is a gene essential for cell wall or cell membrane integrity (claim 105); wherein the essential gene is a gene encoding an enzyme that catalyzes steps in the biosynthesis of DAP (claim 106); wherein the essential gene is an *asd* gene (claim 107).

Galan *et al.* disclose an *asd* mutant (and a method of making said mutant) of *Salmonella* wherein the chromosomal *asd* gene is deleted and the strain contains a plasmid with the *asd* gene (see abstract). The *asd* gene is connected to a P_{trc} promoter, which serves as a means of engineered expression (see page 32, column 1). The instant specification defines a permissive environment as an environment in which the claimed cells are viable, and a non-permissive environment as one in which the cells are non-viable or temporarily viable. As all gene expression would cease when a cell dies (becomes non-viable), the essential gene (*asd*) of the cells disclosed by Galan *et al.* would not be expressed in a non-permissive environment.

Galan *et al.* differs from the instant application in that the essential gene is not linked to *araC*-Pbad and the permissive environment is not disclosed as containing a nutrient required to maintain expression of the essential gene.

Guzman *et al.* disclose plasmid vectors containing the *araC*-Pbad promoter (see abstract). In the presence of arabinose, transcription from this promoter is turned on, and in its absence, transcription only occurs at very low levels (page 4121, column 2, paragraph 2). Guzman *et al.* also teach that the tight control of expression provided by the *araC*-Pbad promoter system is an important characteristic that is mostly absent in other available expression systems, and that this feature has been indispensable in the isolation and study of null mutations in essential genes and in the evaluation of the depletion phenotype of these genes (page 4128, column 2, paragraph 2).

Guzman *et al.* further teach that it is useful to express a cloned gene from an inducible promoter and assess the effect of the expression or depletion of the gene product in mutants lacking the chromosomal gene, and that in these situations, it is highly desirable to use a system (such as the *araC*-Pbad system) that can be efficiently shut off (page 4121, column 1, paragraph 1).

Therefore, it would have been obvious to one of skill in the art to use the *araC*-Pbad promoter system (as disclosed by Guzman *et al.*) to control expression of the *asd* gene in the bacterial cells of Galan *et al.* because it is useful to use such a promoter to control expression in the study of depletion phenotypes.

One would have had a reasonable expectation of success because the *araC*-Pbad promoter system (as disclosed by Guzman *et al.*) has been shown to control expression gene expression in bacterial cells (see abstract).

Claims 61-62, 64, 70-73, 83-86, 87-94, and 104-107 are rejected under 35 U.S.C. 103(a) as being unpatentable over Galan *et al.* (Gene, 94:29-35, 1990) in view of Glick *et al.* (Molecular Biotechnology, Principles and Applications of Recombinant DNA, 1994, ASM Press, pp. 90-92).

The instant claims are drawn to an isolated microbial cell comprising an Environmentally Limited Viability System, wherein the cell is viable in a permissive environment and non-viable or temporarily viable in a non-permissive environment, the system comprising an essential gene (*asd*), wherein expression of the essential gene in the cell is essential to the viability of the cell, the essential gene is expressed when the cell is in the permissive environment and is not expressed or temporarily expressed when the cell is in the non-permissive environment (claim 61); wherein the cell of claim 61 wherein the cell grows in the permissive environment and dies in the non-permissive environment (claim 62); wherein the permissive environment is inside a warm-blooded animal and the non-permissive environment is outside a warm-blooded animal (claims 64, 86, 90); wherein the cell is a gram-negative bacterium (claim 70); wherein the gram-negative bacterium is an enteric bacterium (claim 71); wherein the genus of the enteric bacterium is selected from the group consisting of Escherichia and Salmonella (claim 72); wherein the essential gene is carried on an extrachromosomal vector (claim 73); and wherein the essential gene has engineered expression (claim 83). Additional claims are drawn to a method of making a cell strain with environmentally limited viability comprising stably introducing into a cell an

essential gene (*asd*), wherein expression of the essential gene in the cell is essential to the viability of the cell, the essential gene is expressed when the cell is in the permissive environment and is not expressed or temporarily expressed when the cell is in the non-permissive environment; wherein the cell strain is viable in a permissive environment and non-viable or temporarily viable in a non-permissive environment, and wherein the essential gene is essential for metabolism, growth, cell wall integrity, or cell membrane integrity of the cell (claim 84); wherein the permissive environment comprises a temperature of about 37°C and the non-permissive environment comprises a temperature of less than about 30°C (claims 85 and 89); wherein the cell is temporarily viable in the non-permissive environment (claim 87). Claims are also drawn to an isolated microbial cell comprising an Environmentally Limited Viability System, wherein the cell is viable in a permissive environment and non-viable in a non-permissive environment, the system comprising an essential gene, wherein expression of the gene in the cell is essential to the viability of the cell, and wherein said essential gene is expressed when the cell is in the permissive environment and is not expressed when the cell is in the non-permissive environment, and wherein said essential gene is essential for metabolism, growth, cell wall integrity, or cell membrane integrity of the cell (claim 88); wherein the essential gene is carried on an extrachromosomal vector (claim 91); wherein the cell is a gram-negative bacterium (claim 92); wherein the gram-negative bacterium is an enteric bacterium (claim 93); wherein the genus of the enteric bacterium is selected from the group consisting of Escherichia and Salmonella (claim 94); wherein the essential gene is a gene essential for metabolism or growth of the cell (claim 104); wherein the essential is a gene essential for cell wall or cell membrane integrity (claim 105); wherein the essential gene is a gene encoding an enzyme that catalyzes steps in the biosynthesis of DAP (claim 106); wherein the essential gene is an *asd* gene (claim 107).

Galan *et al.* disclose an *asd* mutant (and a method of making said mutant) of *Salmonella* wherein the chromosomal *asd* gene is deleted and the strain contains a plasmid with the *asd* gene (see abstract). The *asd* gene is connected to a P_{trc} promoter, which serves as a means of engineered expression (see page 32, column 1). The instant specification defines a permissive environment as an environment in which the claimed cells are viable, and a non-permissive environment as one in which the cells are non-viable or temporarily viable. As all gene

expression would cease when a cell dies (becomes non-viable), the essential gene (*asd*) of the cells disclosed by Galan *et al.* would not be expressed in a non-permissive environment.

Galan *et al.* differs from the instant application in that Galan *et al.* do not disclose the permissive environment as inside a warm-blooded animal and the non-permissive environment as outside a warm-blooded animal, or the permissive environment as comprising a temperature of about 37°C and the non-permissive environment as comprising a temperature of less than about 30°C.

Glick *et al.* disclose the regulatable strong promoter *p*L, which is controlled by the *c*I repressor protein of bacteriophage λ. A temperature sensitive mutant of this repressor, cI_{857} is used to regulate expression so that transcription can proceed at 42°C, but not at 30°C (page 91, paragraph 2). It is noted that this is the same repressor system disclosed in the instant specification to allow growth at 37°C (the temperature inside a warm-blooded animal), but not at 30°C (a temperature outside a warm-blooded animal). Glick *et al.* further teach that the use of regulatable strong promoters is advantageous, and that it is desirable to control transcription in such a way that a cloned gene is expressed only at a specific stage in the host cell growth cycle and only for a specified duration, because a high level of continual expression of a cloned gene is often detrimental to the host cell (page 90, paragraph 2).

Therefore, it would have been obvious to one of skill in the art to use the temperature-regulated *p*L promoter system (as disclosed by Glick *et al.*) to control expression of the *asd* gene in the bacterial cells of Galan *et al.* because regulatable strong promoters are advantageous to avoid a high level of continual expression of a cloned gene which is often detrimental to the host cell.

One would have had a reasonable expectation of success because the use of the temperature-regulated *p*L promoter system in order to control gene expression (as disclosed by Glick *et al.*) is well known in the art (as evidenced by the fact that Glick *et al.* is a textbook).

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The instant claims are drawn to an isolated microbial cell comprising an Environmentally Limited Viability System, wherein the cell is viable in a permissive environment and non-viable or temporarily viable in a non-permissive environment, the system comprising an essential gene (*asd*), wherein expression of the essential gene in the cell is essential to the viability of the cell, the essential gene is expressed when the cell is in the permissive environment and is not expressed or temporarily expressed when the cell is in the non-permissive environment (claim 61); wherein the cell of claim 61 wherein the cell grows in the permissive environment and dies in the non-permissive environment (claim 62); wherein the cell is a gram-negative bacterium (claim 70); wherein the gram-negative bacterium is an enteric bacterium (claim 71); wherein the genus of the enteric bacterium is selected from the group consisting of Escherichia and Salmonella (claim 72); wherein the essential gene is carried on an extrachromosomal vector (claim 73); and wherein the essential gene has engineered expression (claim 83). Additional claims are drawn to a method of making a cell strain with environmentally limited viability comprising stably introducing into a cell an essential gene (*asd*), wherein expression of the essential gene in the cell is essential to the viability of the cell, the essential gene is expressed when the cell is in the permissive environment and is not expressed or temporarily expressed when the cell is in the non-permissive environment; wherein the cell strain is viable in a permissive environment and non-viable or temporarily viable in a non-permissive environment, and wherein the essential gene is essential for metabolism, growth, cell wall integrity, or cell membrane integrity of the cell (claim 84). Claims are also drawn to an isolated microbial cell comprising an Environmentally Limited Viability System, wherein the cell is viable in a permissive environment and non-viable in a non-permissive environment, the system comprising an essential gene, wherein expression of the gene in the cell is essential to the viability of the cell, and wherein said essential gene is expressed when the cell is in the permissive environment

and is not expressed when the cell is in the non-permissive environment, and wherein said essential gene is essential for metabolism, growth, cell wall integrity, or cell membrane integrity of the cell (claim 88); wherein the essential gene is carried on an extrachromosomal vector (claim 91); wherein the cell is a gram-negative bacterium (claim 92); wherein the gram-negative bacterium is an enteric bacterium (claim 93); wherein the genus of the enteric bacterium is selected from the group consisting of Escherichia and Salmonella (claim 94); wherein the essential gene is a gene essential for metabolism or growth of the cell (claim 104); wherein the essential is a gene essential for cell wall or cell membrane integrity (claim 105); wherein the essential gene is a gene encoding an enzyme that catalyzes steps in the biosynthesis of DAP (claim 106); wherein the essential gene is an *asd* gene (claim 107).

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chromosomal gene, and that in these situations, it is highly desirable to use a system (such as the *araC*-Pbad system) that can be efficiently shut off (page 4121, column 1, paragraph 1).

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bacterium is an enteric bacterium (claim 93); wherein the genus of the enteric bacterium is selected from the group consisting of Escherichia and Salmonella (claim 94); wherein the essential gene is a gene essential for metabolism or growth of the cell (claim 104); wherein the essential is a gene essential for cell wall or cell membrane integrity (claim 105); wherein the essential gene is a gene encoding an enzyme that catalyzes steps in the biosynthesis of DAP (claim 106); wherein the essential gene is an *asd* gene (claim 107).

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in the bacterial cells of Curtiss because regulatable strong promoters are advantageous to avoid a high level of continual expression of a cloned gene which is often detrimental to the host cell.

One would have had a reasonable expectation of success because the use of the temperature-regulated *p*L promoter system in order to control gene expression (as disclosed by Glick *et al.*) is well known in the art (as evidenced by the fact that Glick *et al.* is a textbook).

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The instant claims are drawn to an isolated microbial cell comprising an Environmentally Limited Viability System, wherein the cell is viable in a permissive environment and non-viable or temporarily viable in a non-permissive environment, the system comprising an essential gene (*asd*), wherein expression of the essential gene in the cell is essential to the viability of the cell, the essential gene is expressed when the cell is in the permissive environment and is not expressed or temporarily expressed when the cell is in the non-permissive environment (claim 61); wherein the cell of claim 61 wherein the cell grows in the permissive environment and dies in the non-permissive environment (claim 62); wherein the cell is a gram-negative bacterium (claim 70); wherein the gram-negative bacterium is an enteric bacterium (claim 71); wherein the genus of the enteric bacterium is selected from the group consisting of Escherichia and Salmonella (claim 72); wherein the essential gene is carried on an extrachromosomal vector (claim 73); and wherein the essential gene has engineered expression (claim 83). Additional claims are drawn to a method of making a cell strain with environmentally limited viability comprising stably introducing into a cell an essential gene (*asd*), wherein expression of the essential gene in the cell is essential to the viability of the cell, the essential gene is expressed

when the cell is in the permissive environment and is not expressed or temporarily expressed when the cell is in the non-permissive environment; wherein the cell strain is viable in a permissive environment and non-viable or temporarily viable in a non-permissive environment, and wherein the essential gene is essential for metabolism, growth, cell wall integrity, or cell membrane integrity of the cell (claim 84). Claims are also drawn to an isolated microbial cell comprising an Environmentally Limited Viability System, wherein the cell is viable in a permissive environment and non-viable in a non-permissive environment, the system comprising an essential gene, wherein expression of the gene in the cell is essential to the viability of the cell, and wherein said essential gene is expressed when the cell is in the permissive environment and is not expressed when the cell is in the non-permissive environment, and wherein said essential gene is essential for metabolism, growth, cell wall integrity, or cell membrane integrity of the cell (claim 88); wherein the essential gene is carried on an extrachromosomal vector (claim 91); wherein the cell is a gram-negative bacterium (claim 92); wherein the gram-negative bacterium is an enteric bacterium (claim 93); wherein the genus of the enteric bacterium is selected from the group consisting of Escherichia and Salmonella (claim 94); wherein the essential gene is a gene essential for metabolism or growth of the cell (claim 104); wherein the essential is a gene essential for cell wall or cell membrane integrity (claim 105); wherein the essential gene is a gene encoding an enzyme that catalyzes steps in the biosynthesis of DAP (claim 106); wherein the essential gene is an *asd* gene (claim 107).

Curtiss discloses an *asd* mutant (and a method of making said mutant) of *Salmonella* wherein the chromosomal *asd* gene is deleted and the strain contains a plasmid with the *asd* gene (col. 8, line 66- col. 10. line 11; claims 1, 3, and 7-8). The instant specification defines a permissive environment as an environment in which the claimed cells are viable, and a non-permissive environment as one in which the cells are non-viable or temporarily viable. As all gene expression would cease when a cell dies (becomes non-viable), the essential gene (*asd*) of the cells disclosed by Curtiss would not be expressed in a non-permissive environment.

Curtiss differs from the instant application in that Curtiss does not disclose the permissive environment as inside a warm-blooded animal and the non-permissive environment as outside a warm-blooded animal, or the permissive environment as comprising a temperature of about 37°C and the non-permissive environment as comprising a temperature of less than about 30°C.

Glick *et al.* (Molecular Biotechnology, Principles and Applications of Recombinant DNA, 1994, ASM Press, pp. 90-92) disclose the regulatable strong promoter *p*L, which is controlled by the *c*I repressor protein of bacteriophage λ. A temperature sensitive mutant of this repressor, cI_{857} is used to regulate expression so that transcription can proceed at 42°C, but not at 30°C (page 91, paragraph 2). It is noted that this is the same repressor system disclosed in the instant specification to allow growth at 37°C (the temperature inside a warm-blooded animal), but not at 30°C (a temperature outside a warm-blooded animal). Glick *et al.* further teach that the use of regulatable strong promoters is advantageous, and that it is desirable to control transcription in such a way that a cloned gene is expressed only at a specific stage in the host cell growth cycle and only for a specified duration, because a high level of continual expression of a cloned gene is often detrimental to the host cell (page 90, paragraph 2).

Therefore, it would have been obvious to one of skill in the art to use the temperature-regulated *p*L promoter system (as disclosed by Glick *et al.*) to control expression of the *asd* gene in the bacterial cells of Curtiss because regulatable strong promoters are advantageous to avoid a high level of continual expression of a cloned gene which is often detrimental to the host cell.

One would have had a reasonable expectation of success because the use of the temperature-regulated *p*L promoter system in order to control gene expression (as disclosed by Glick *et al.*) is well known in the art (as evidenced by the fact that Glick *et al.* is a textbook).

Conclusion

No claim is allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Brian J. Gangle whose telephone number is (571) 272-1181. The examiner can normally be reached on M-F 7-3:30.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Jeffrey Siew can be reached on (571) 272-0787. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

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